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ORIGINAL ARTICLE

Recombinant OmpA protein fragments mediate interleukin-17 regulation to prevent *Escherichia coli* meningitis

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bacterial meningitis;
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Abstract *Background:* Neonates are at a higher risk for bacterial meningitis than children of other age groups. Although the mortality rates have decreased over the past few decades, neonatal meningitis is still a severe disease with high morbidity. For bacterial meningitis, antibiotic therapy is the primary choice for management. However, neurologic complications often cannot be averted; ~40% of survivors exhibit neurological sequelae. *Escherichia coli* infection is the common cause of neonatal meningitis. Previously, we have demonstrated that the recombinant loop 1–3, loop 2–3, and loop 2–4 fragments of OmpA protein can protect mice from death after intracerebral *E. coli* infection. In this study, the protective effects of the recombinant OmpA protein fragments in *E. coli* intracerebral infections were investigated.
Methods: The effects of *E. coli* intracerebral infection on cytokine and chemokine expression were determined. We also used various recombinant fragments of the OmpA protein to investigate the effects of these recombinant OmpA protein fragments on cytokine and chemokine expression.
Results: In this study, we demonstrated that the expression of interleukin-17 and other cytokines, chemokines, inducible nitric oxide synthase, and cyclooxygenase-2 are involved in the inflammatory processes of intracerebral *E. coli* infection. We also demonstrated that specific recombinant OmpA protein fragments (L1–3, L2–3, L2–4, and L3) can regulate cytokine, chemokine, nitric oxide synthase, and cyclooxygenase-2 expression and, subsequently, protect mice from death caused by intracerebral infection of *E. coli*.

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Conclusion: This finding indicates the potential for developing a new therapeutic approach to improve the prognosis of bacterial meningitis.

Introduction

A 29-year assessment of the evolving trends in neonatal and childhood bacterial meningitis in Northern Taiwan revealed that *Escherichia coli* and *Streptococcus agalactiae* (Group B *Streptococcus*) are the two most common pathogens of neonatal bacterial meningitis. In the past 5 years (2008–2012), *E. coli* has surpassed Group B *Streptococcus*, becoming the most common pathogen of neonatal meningitis.[1] *E. coli* strains with K1 capsular polysaccharide are the most predominant strains associated with neonatal bacterial meningitis.[2] Several K1-associated components including Fim H, K1 capsule, and outer membrane protein A (OmpA) participate in brain microvascular endothelial cell (BMEC) binding and invasion.[3–7] OmpA is the determinant of *E. coli* that mediates host–cell interactions and is crucial for the invasion of *E. coli* K1 into macrophages and monocytes.[8] The OmpA protein is also the major component enabling *E. coli* to adhere to and invade into BMECs and astrocytes,[5,9–11] and this binding is the most critical step in *E. coli* K1 infection.[11,12] In addition, OmpA is highly conserved through the evolution of gram-negative bacilli. This protein is the receptor for several bacteriophages,[13–16] and is essential in bacterial conjugation.[17–19] OmpA is encoded using a 1038 bp open reading frame comprising a 21-amino-acid leader peptide and a mature 325-amino-acid protein. The N-terminal membrane-anchoring domain of OmpA is essential for effective folding and function. The N-terminal domain forms an antiparallel β-barrel, containing eight transmembrane β-strands, connected by three periplasmic turns, and four surface-exposed hydrophilic loops. The OmpA C-terminal domain interacts with the peptidoglycan layer in the periplasm to maintain outer membrane integrity.[20] Purified N-terminal amino acids 1–171 of OmpA bind directly to BMECs, whereas a derivative lacking all four extracellular loops cannot.[6] In addition, bacterial entry into BMECs is governed by loops 1, 2, and 3.[21] Specifically, the short peptides corresponding to loops 1 and 2 can block the OmpA$^+$ *E. coli* invasion of BMECs.[9] In addition, the OmpA regions in loops 1, 2, and 4 resist serum bactericidal activity through increased binding to a complement regulatory protein, C4b-binding protein. The OmpA regions in loops 1 and 2 are responsible for *E. coli* survival in tissues and blood, resulting in high-grade bacteremia, which is a prerequisite for the onset of meningitis.[22]

Inflammatory molecules such as cytokines, chemokines, inducible nitric oxide synthase (NOS-2), and cyclooxygenase-2 (COX-2) mediate a large amount of the neurologic damage in central nervous system (CNS) infections.[12,23] CD4+ T cells differentiate into two subsets: CD4+ T helper 1 and T helper 2 cells. T helper 1 cells producing interleukin (IL)-2 and interferon γ (IFN-γ) induce cell-mediated immunity, whereas T helper 2 cells secreting IL-4, IL-5, and IL-13 stimulate humoral immunity.[24] T helper 17 cells producing IL-17 represent a subset distinct from other CD4+ Tcell subsets; they provide protection against some infections and are associated with the development of autoimmune diseases. IL-17 functions as a proinflammatory cytokine; can recruit granulocyte lineage, especially neutrophils[25]; and functions synergistically with other inflammatory stimuli in the CNS. IL-17 was required for the efficient eradication of bacterial infection, stimulating granulopoiesis, neutrophil recruitment, and proinflammatory molecules production.[26]

We previously reported that the recombinant full-length, loop 1–3, loop 2–3, and loop 2–4 fragments of the OmpA protein can protect mice from death after intracerebral *E. coli* RS218 infection.[11,27] In this study, the protective effects of the recombinant OmpA protein fragments in *E. coli* RS218 intracerebral infections were investigated.

Materials and methods

Chemicals, bacteria culture, and cell culture

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated. The recombinant OmpA protein fragments (loops 1–2, 1–3, 1–4, 2–3, 2–4, and 3–4) were expressed and purified as described in our previous report.[27] Briefly, the DNA fragments were amplified and ligated into the pET-21a expression vector (Novagen, Darmstadt, Germany). The resultant plasmid was transformed into *E. coli* BL21 (DE3). Protein expression was induced and then purified using Ni^{2+}-charged sepharose according to the manufacturer's instructions (GE Healthcare Life Science, WI, USA). The recombinant OmpA protein fragments loop 2 (amino acids 82–92) and loop 3 (amino acids 128–134) were synthesized by Genomics Bioscience & Technology Co., (Taipei, Taiwan). The *E. coli* strains used in the present study were kindly provided by Dr. K.S. Kim (Division of Pediatric Infectious Diseases, School of Medicine, Johns Hopkins University, Baltimore, MD, USA); RS218 (O18:K1:H7) was isolated from the cerebrospinal fluid of a neonate with meningitis. E91 is an RS218 mutant that lacks the entire *ompA* gene.[9] The bacteria were grown in a brain heart infusion broth with appropriate antibiotics (Difco Laboratories, Detroit, MI, USA). For infection experiments, overnight cultures were expanded in the brain heart infusion broth and incubated at 37°C for 2–3 hours to the midlog phase. The bacteria were centrifuged at 10,000*g* for 5 minutes and resuspended in a cell culture medium

without antibiotics. Cell Line C6, a rat glioma cell line (ATCC CCL-107), was purchased from Bioresource Collection and Research Center, Hsinchu, Taiwan. Cells were cultured in Ham F10 medium with 15% horse serum, 2.5% fetal bovine serum, and 50 U/mL of penicillin-streptomycin (all from Life Technologies, Gaithersburg, MD, USA).

Reverse transcription-polymerase chain reaction and multiplex polymerase chain reaction

Confluent monolayer C6 cells were incubated with *E. coli* RS218 at multiplicity of infection of 10 for 2 hours at 37°C. The cells were washed with culture medium three times and incubated with culture medium containing 100 μg/mL of gentamicin (Life Technologies) for 2 hours to kill the extracellular bacteria. The cells were washed three times with *phosphate-buffered saline* (PBS) and RNA was extracted as described by the manufacturer with minor modifications (Trizol reagent, Invitrogen). The extracted RNA sample was immediately used for reverse transcription (RT)-polymerase chain reaction (PCR), using a one-step RT-PCR kit as described by the manufacturers (Qiagen, Valencia, CA, USA). Then multiplex (M) PCR reactions were set up using a MPCR Kit for Rat Sepsis Cytokines Set-2 as described by the manufacturers (MBI, San Francisco, CA, USA).

Animal experiments

C57BL/6 mice were obtained from the National Laboratory Animal Center of Taiwan (Nangang, Taipei, Taiwan) and maintained under pathogen-free conditions. This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Taipei Medical University, Taipei, Taiwan. The protocol was approved by the Institutional Animal Care and Use Committee of the Taipei Medical University (Approval IDs: LAC-99-0009). All surgery was performed under pentobarbital sodium salt anesthesia, and every effort was made to minimize suffering. In this experiment, male, 8—12-week-old C57BL/6 mice were randomly distributed into groups. The experimental protocol developed by Tsao et al[12] was followed to assess the survival of mice after intracerebral bacterial administration. Each group containing five mice were anesthetized with pentobarbital sodium salt (50 mg/kg) with intraperitoneal injection; each brain was then infected with *E. coli* RS218 (5×10^5 in 20 μL of PBS). Concurrently, PBS (20 μL) was used as a negative control. The mice were monitored for survival every 12 hours for 8 days. To investigate the effects of the recombinant OmpA protein fragments on the survival of the C57BL/6 mice after intracerebral *E. coli* RS218 administration, each brain was infected with *E. coli* RS218 (5×10^5 in 20 μL of PBS) with intracerebral injection in the presence of each recombinant OmpA protein fragment (20 μg); the survival of these mice was observed for up to 8 days after administration. Groups of three C57BL/6 mice were infected with *E. coli* RS218 with intracerebral injection in the presence or absence of each recombinant OmpA protein fragment. The mice were killed at various time points after the challenge. For cytokine measurements, the brains were removed and stored at −80°C and performed according to the manufacturer's instructions of 23-plexpanel bead-based immunoassay kits (M60-009RDPD, Bio-Rad, Hercules, CA, USA). For analyses, samples were thawed in 1.5 mL of cell lysis buffer (Bio-Rad) containing a protease inhibitor cocktail and 3 μL of a stock solution containing 500mM phenylmethylsulfonyl fluoride in dimethyl sulfoxide (Sigma, St. Louis, MO, USA). Samples were homogenized by drawing samples up and down through a 1 mL pipette tip (cut back to a 2 mm opening) 20 times. Then, the samples were centrifuged at 4500*g* for 15 minutes at 4°C and supernatants were collected. The total protein concentration was determined using a Protein Assay Kit (Bio-Rad). All tissue samples were diluted with cell lysis buffer as needed to a final total protein concentration of 500 μg/mL. For Western blotting, brains were homogenized with a protein extraction buffer (50 mmol/L of Tris HCl, pH 8.0, 150 mmol/L of NaCl, 1% NP-40, and the protease inhibitor cocktails (Bio-Rad), including 0.2 mmol/L of phenylmethanesulfonyl fluoride, 20 μg/mL of aprotinin, and 20 μg/mL of leupeptin and harvested for whole cell lysate. For remnant bacteria detection, brains were aseptically removed and homogenized with 3% gelatin in PBS. The samples were serially diluted, and the colony forming unit for each sample was determined on a blood agar plate.

Cytokine measurements

Brain homogenates were assayed for cytokines using 23-plexpanel bead-based immunoassay kits [including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor, IFN-γ, keratinocyte-derived cytokine (KC), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, RANTES (regulated on activation, normal T cell expressed and secreted), tumor necrosis factor (TNF)-α] (M60-009RDPD, Bio-Rad), and performed according to the manufacturer's instructions. Cytokine data were analyzed using Bio-Plex Manager 3.0 software (Bio-Rad).

Electrophoresis and immunoblotting

Electrophoresis was performed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Reagents for odium dodecyl sulfate-polyacrylamide gel electrophoresis were from Bio-Rad. After electrophoresis, proteins on the gel were electrotransferred onto a polyvinylidene fluoride (PVDF) membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). After transfer, the PVDF membranes were blocked with blocking solution containing 5% skim milk in TBST (10 mmol/L Tris, pH7.5, 100 mmol/L NaCl, and 0.1% Tween 20) for 1 hour at room temperature. Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) unless otherwise indicated. The PVDF membrane was incubated with a solution containing rabbit anti-NOS-2 antibody (1:200), rabbit anti-COX-2 antibody (1:200), or goat antiactin antibody (1:200) in the blocking buffer for 2 hours. After washing, the PVDF membrane was incubated with peroxidase-linked secondary antibody (1:2000) for 1

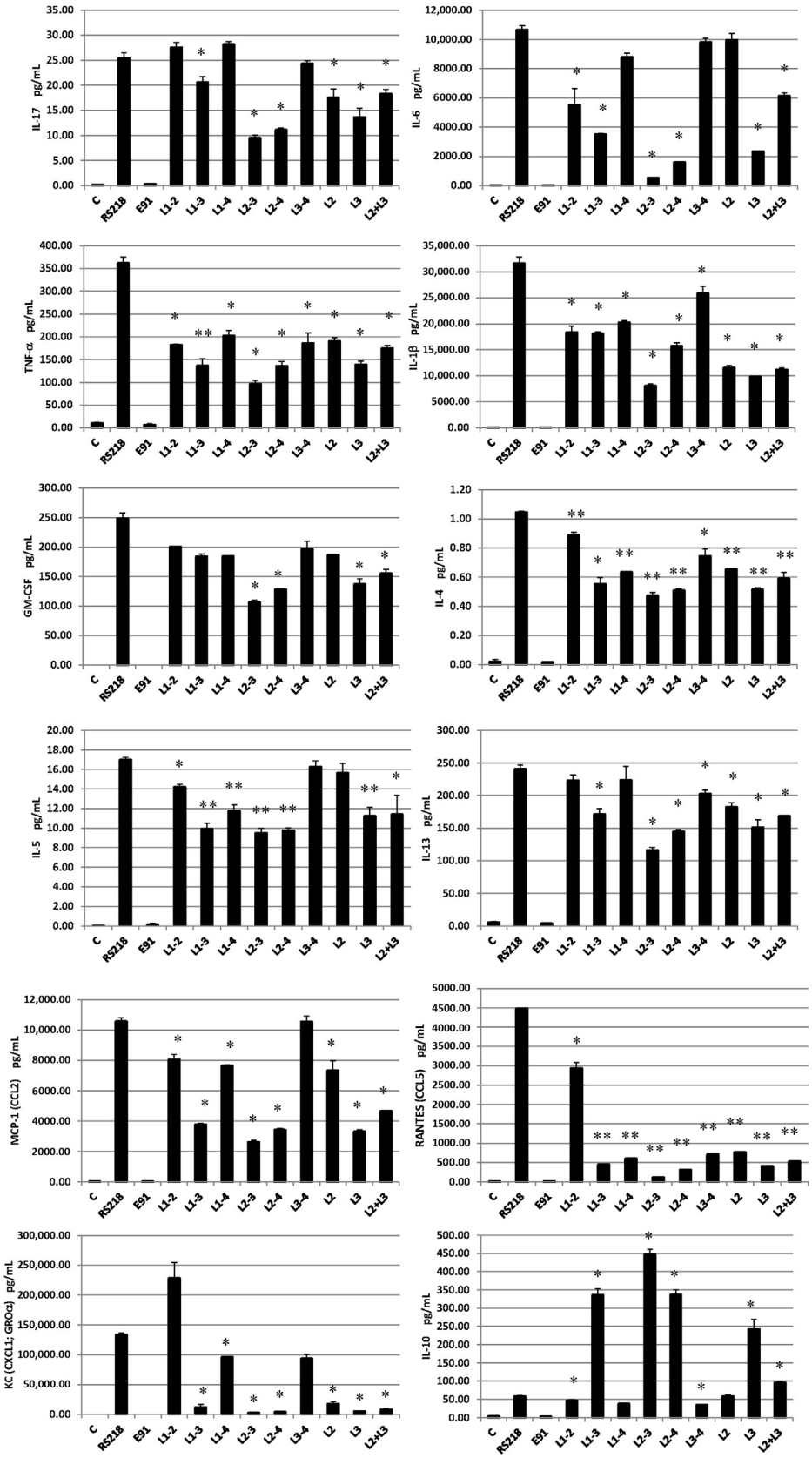

Figure 1. Intracerebral infection of *Escherichia coli* RS218 induced the expression of various cytokines and chemokines *in vivo*. Groups of three mice were anesthetized and challenged using intracerebral injection with 5×10^5 bacteria alone or a premixture of 20 μg of each recombinant OmpA protein fragment: L1—2, L1—3, L1—4, L2—3, L2—4, L3—4, L2, L3, and L2 + L3. The brains were collected after 48 hours of treatment. Individual cytokines and chemokines were quantified. Paired Student *t* tests were used to

hour. Finally, the PVDF membrane was developed using a chemiluminescence kit (Amersham).

Statistical analysis

The results were expressed as the mean ± standard deviation of independent experiments. Quantitative variables were analyzed using the paired Student *t* test. The results were considered significant when the calculated $p < 0.05$.

Results

Intracerebral injection of *E. coli* RS218 induced the expression of IL-17 and other cytokines and chemokines; recombinant OmpA protein fragments regulated this expression

Inflammatory molecules such as cytokines mediate a large amount of the neurologic damage in CNS infections.[12,23] The effects of *E. coli* RS218 intracerebral infection on cytokine and chemokine expression were determined. We challenged the mice by using intracerebral injection to avoid interference with the blood–brain barrier (BBB) according to the protocol presented by Tsao et al.[12] Groups of three C57BL/6 mice were anesthetized and then infected with an intracerebral injection of *E. coli* RS218 or E91 (an RS218 mutant that lacks the entire *ompA* gene) for 24 hours. The brains were removed, and homogenates were assayed for 23 cytokines and chemokines. The results showed that intracerebral injection of *E. coli* RS218 but not E91 induced the expression of IL-17, IL-6, TNF-α, IL-1β, GM-CSF, IL-4, IL-5, IL-13, and chemotactic chemokines, namely MCP-1 (CCL2), RANTES (CCL5), and KC (CXCL1; GROα) (Figure 1). In addition, we determined whether the presence of recombinant OmpA protein fragments regulated cytokine or chemokine expression in *E. coli* meningitis. Groups of three C57BL/6 mice were challenged by intracerebrally injecting them with a mixture of *E. coli* RS218 and 20 μg of each recombinant OmpA protein fragment (loops 1–2, 1–3, 1–4, 2–3, 2–4, 3–4, L2, L3, and L2 + L3), and the cytokine expression was measured. Individual recombinant OmpA protein fragments exerted various effects on the cytokine and chemokine expression (Figure 1). The recombinant OmpA protein fragments L1–3, L2–3, L2–4, and L3 greatly inhibited the expression of IL-17, IL-6, TNF-α, IL-1β, GM-CSF, IL-4, IL-5, IL-13, and the chemotactic chemokines MCP-1 (CCL2), RANTES (CCL5), and KC (CXCL1; GROα). By contrast, the recombinant OmpA protein fragments L1–3, L2–3, L2–4, and L3 upregulated the expression of IL-10, an immunosuppressive cytokine.

In a previous study, we identified that OmpA-mediated *E. coli* infection activates astrocytes *in vitro* and in the mouse brain.[11] In addition, cytokine induction after *E. coli* RS218 ($OmpA^+$) infection in astrocytes was determined using RT-PCR and MPCR. We employed rat glioma C6 cells[28,29] as an *in vitro* system of astrocytes. After the cells had been infected with *E. coli* RS218 for 2 hours, the expression of IL-6, TNF-α, and IL-1β mRNA was induced at individual time intervals (Figure 2).

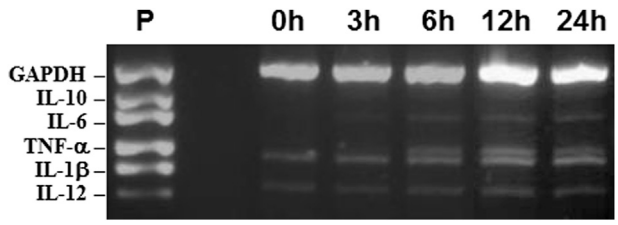

Figure 2. Cytokine expression in astrocytes induced by *Escherichia coli* RS218 infection. When the C6 cells had been infected with *E. coli* RS218 for 2 hours, the total mRNA of the C6 cells was extracted, and the cytokine expression was then determined using reverse transcriptase-polymerase chain reaction and multiplex polymerase chain reaction. The expression of interluekin-6, tumor necrosis factor-α, and interluekin-1β mRNA was induced in C6 cells infected by *E. coli* RS218. Glyceraldehyde 3-phosphate dehydrogenase was used as a loading control. GADPH = glyceraldehyde 3-phosphate dehydrogenase; IL = interleukin; TNF = tumor necrosis factor.

Recombinant OmpA protein fragments inhibited *E. coli*-RS218-infection-induced iNOS-2 and COX-2 expression

The inflammatory molecules NOS-2 and COX-2 play crucial roles in the neurologic damage of CNS infections[12,23]; the expression of NOS-2 and COX-2 after *E. coli* RS218 infection was examined. Groups containing three mice each were infected with bacteria through intracerebral injection. At 24 hours postchallenge, the brains were removed and analyzed for NOS-2 and COX-2 expression by conducting immunoblotting. As shown in Figures 3A and 3B, NOS-2 and COX-2 expression was induced after infection with *E. coli* RS218. In addition, whether the presence of recombinant OmpA protein fragments inhibited NOS-2 and COX-2 expression in the mouse brains was determined. Additional groups of mice were challenged by intracerebrally injecting them with a mixture of *E. coli* RS218 and 20 μg of each recombinant OmpA protein fragment (loops 1–2, 1–3, 1–4, 2–3, 2–4, 3–4, L2, L3, and L2 + L3); NOS-2 and COX-2 expression was then analyzed by performing immunoblotting. The results showed that the NOS-2 and COX-2 expression in the brain was inhibited by the recombinant OmpA protein fragments L1–3, L2–3, L2–4, L2, L3, and L2 + L3 (Figures 3A and 3B). Taken together, these data indicated that specific recombinant OmpA protein fragments (L1–3, L2–3, L2–4, and L3) protected the mice from the expression of NOS-2, COX-2, cytokines, and chemokines in the brain. These effects may have protected the mice from severe neuronal damage.

compare the RS218 results (*$p < 0.05$; **$p < 0.001$). GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; KC = mouse keratinocyte-derived cytokine; MCP = monocyte chemoattractant protein; TNF = tumor necrosis factor.

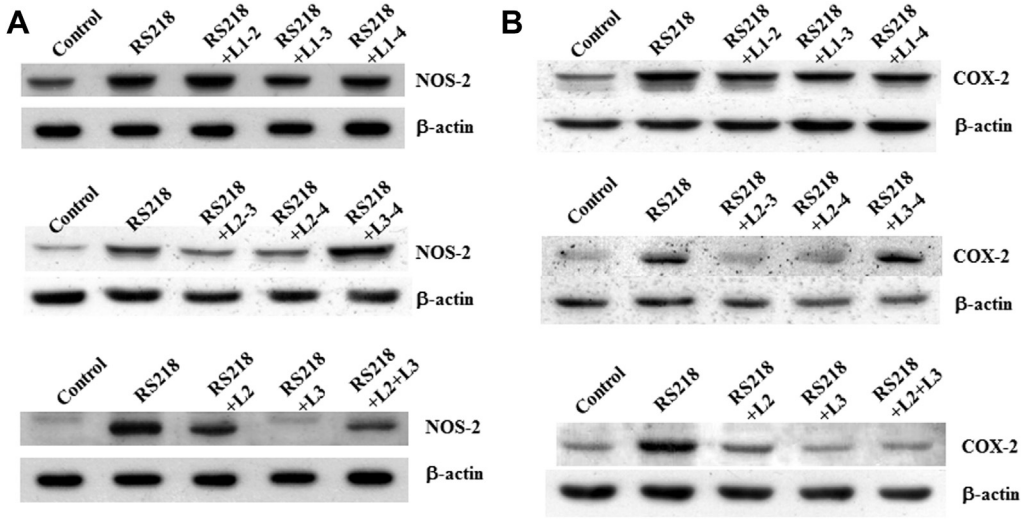

Figure 3. Specific recombinant OmpA protein fragments inhibited the nitric oxide synthase-2 and cyclooxygenase-2 expression induced by *Escherichia coli* RS218 infection *in vivo*. Groups of three C57BL/6 mice each were anesthetized and challenged by intracerebrally injecting them with 5×10^5 bacteria alone or a premixture of 20 μg of each recombinant OmpA protein fragment: L1—2, L1—3, L1—4, L2—3, L2—4, L3—4, L2, L3, and L2 + L3. The mice were sacrificed 24 hours postinfection. Western analysis of whole brain lysates was performed to detect nitric oxide synthase-2 (A) or cyclooxygenase-2 (B) and actin. COX-2 = cyclooxygenase-2; NOS-2 = nitric oxide synthase-2.

Recombinant OmpA protein fragments protected C57BL/6 mice from death induced by *E. coli* infection

Groups containing five C57BL/6 mice were anesthetized and then infected through intracerebral injection of *E. coli* RS218, and the survival of these mice was assessed for 8 days. The mice died within 48 hours of intracerebral injection of the OmpA$^+$ *E. coli* strain RS218. Next, we determined whether the presence of recombinant OmpA protein fragments protected the mice from *E. coli* RS218 infection. Groups of five C57BL/6 mice were challenged by intracerebrally injecting them with a mixture of *E. coli* RS218 and 20 μg of each recombinant OmpA protein fragment (loops 1—2, 1—3, 1—4, 2—3, 2—4, 3—4, L2, L3, and L2 + L3) and assessed for 8 days. As shown in Figure 4, 50% of the mice injected with *E. coli* RS218 and L1—3, 60% of the mice injected with *E. coli* RS218 and L2—3, 50% of the mice injected with *E. coli* RS218 + L2—4, 50% of the mice injected with *E. coli* RS218 and L3, and 38% of the mice with *E. coli* RS218, L2, and L3 survived until Day 8 postinfection. These results suggested that the recombinant OmpA protein fragments L1—3, L2—3, L2—4, and L3 significantly prolonged the survival of the C57BL/6 mice after intracerebral *E. coli* RS218 infection ($p < 0.05$).

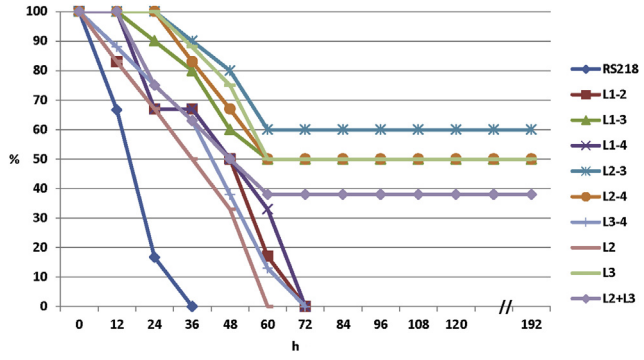

Figure 4. Survival of C57BL/6 mice after infection with *Escherichia coli* RS218. Groups of five C57BL/6 mice were anesthetized and challenged by intracerebrally injecting them with 5×10^5 bacteria alone or a premixture of 20 μg of each recombinant OmpA protein fragment: L1—2, L1—3, L1—4, L2—3, L2—4, L3—4, L2, L3, and L2 + L3. The mice were monitored for survival for up to 8 days.

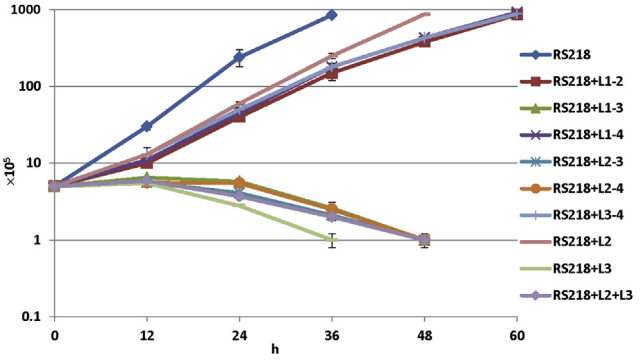

Figure 5. Remnant bacteria in the brain after *Escherichia coli* RS218 infection. Groups of three mice were anesthetized and challenged by intracerebrally injecting them with 5×10^5 bacteria alone or a premixture of 20 μg of each recombinant OmpA protein fragment: L1—2, L1—3, L1—4, L2—3, L2—4, L3—4, L2, L3, and L2 + L3. The brains were collected at various time points after treatment. Remnant bacteria in the brains were quantified.

Recombinant OmpA protein fragments protected the C57BL/6 mice from infection-induced death correlating with bacterial clearance

We examined the remnant bacteria in the brain after *E. coli* RS218 infection, which gradually increased to $(8.5 \pm 0.3) \times 10^7$ at 36 hours postinfection (Figure 5); the mice died within 48 hours of intracerebral *E. coli* RS218 infection. In addition, for mice infected with the *E. coli* RS218 premixed with the recombinant OmpA protein fragments L1—2, L1—4, and L3—4, the remnant bacteria in the brain gradually increased to $(8.6 \pm 0.1) \times 10^7$, $(9.2 \pm 0.0) \times 10^7$, and $(8.8 \pm 0.1) \times 10^7$, respectively, at 60 hours postinfection (Figure 5). Similarly, for the mice infected with *E. coli* RS218 premixed with the recombinant OmpA protein fragment L2, the remnant bacteria in the brain gradually increased to $(8.7 \pm 0.1) \times 10^7$ at 48 hours postinfection. Those mice died within 72 hours after bacterial infection. By contrast, the remnant bacteria in the brains infected with *E. coli* RS218 premixed with the recombinant OmpA protein fragments L1—3, L2—3, L2—4, L3, and L2 + L3 decreased after infection and were undetectable at 48 hours after infection (Figure 5). The results indicated that these specific recombinant OmpA protein fragments protected the mice from *E. coli* infection, and this protection correlated with bacterial clearance.

Discussion

A neuronal injury associated with bacterial infection of the CNS involves multiple microbial and host factors. Severe bacteremia and invasion through BMECs are the determining factors contributing to CNS infection.[30] Despite the availability of advanced antibiotic therapy and patient care for bacterial meningitis; it is still associated with a relatively high morbidity and mortality. Nearly 40% of surviving patients undergo various neurological complications.[1,31,32] These findings indicate that a new therapeutic approach must be developed to improve the prognosis of bacterial meningitis.

Inflammation in the CNS is controlled by various cytokines. In the present study, intracerebral injection of *E. coli* RS218 induced the expression of IL-17, IL-6, TNF-α, IL-1β, GM-CSF, IL-4, IL-5, IL-13, and the chemotactic chemokines MCP-1 (CCL2), RANTES (CCL5), and KC (CXCL1; GROα) (Figure 1). In the CNS, astrocytes and microglia express IL-17 receptors, whereas neurons do not.[33] IL-17 was shown to stimulate the IL-6 and IL-8 released by glioblastoma cells of the CNS.[34] Kawanokuchi et al[33] showed that treatment with IL-17 upregulated the microglial production of IL-6, macrophage inflammatory protein-2, adhesion molecules, neurotrophic factors, and NO.[33] In this study, we demonstrated that *E. coli* RS218 infection induces the expression of IL-1β, IL-6, and TNF-α mRNA in astrocytes (Figure 2).

The chemokine MCP-1 (CCL2) was established as a mediator of inflammation within and outside the CNS and as a stimulator of the extravasation of mononuclear leukocytes into the CNS.[35] Astrocyte-derived MCP-1 regulates BBB integrity and leukocyte penetration of the CNS parenchyma, whereas endothelial-derived MCP-1 affects leukocyte transendothelial migration.[36] Trajkovic et al[37] showed that IL-17 synergized with IFN-γ, IL-1, and TNF-α, thus activating NOS-2 and subsequent NO production in rodent astrocytes. NOS-2 is produced in the CNS in response to inflammation, possibly by astrocytes and microglia as well as by macrophages that invade the CNS. In this study, we showed that intracerebral injection of *E. coli* RS218 induced the expression of NOS-2 and COX-2 (Figures 3A and 3B). Inflammatory processes resulted in the destruction of the BBB, neuron damage, and finally, death in C57BL/6 mice. Our data demonstrated that specific recombinant OmpA protein fragments (L1—3, L2—3, L2—4, and L3) can inhibit the expression of cytokines, chemokines, NOS-2, and COX-2 *in vivo*. The same recombinant OmpA protein fragments also upregulate the expression of IL-10, an immunosuppressive cytokine, thus preventing subsequent inflammatory processes. The counteraction between the proinflammatory cytokines and protective cytokines determines the final outcome in organ and tissues. We have previously demonstrated that the recombinant full-length, L1—3, L2—3, and L2—4 fragments of the OmpA protein can protect mice from death after intracerebral *E. coli* infection.[11,27] Furthermore, based on the results of the present study, we propose that L2—3 and L3 fragments of the OmpA protein may act as the shorter protective peptides that can regulate the expression of cytokines, chemokines, NOS-2, and COX-2 and, subsequently, protect mice from death caused by the intracerebral injection of *E. coli* RS218. In bacterial meningitis, although antibiotic therapy is the primary choice for management, neurological complications can seldom be averted. Thus, the possible protective effects of recombinant OmpA protein fragments on patients with bacterial meningitis as well as the routes for administering these fragments require further investigation.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

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